

Supplementary File 2

For the calculation of Average Nucleotide Identity (ANI) values, the program, JSpecies (Richter and Rosselló-Móra, 2009), was employed. Pair-wise comparisons between genomes were made by artificially sectioning each query genome into fragments consisting of 1020 nucleotides and then comparing these sections to the reference genome (Goris et al., 2007, Richter and Rosselló-Móra, 2009) using BLAST. Fragments with more than 30% identity, as calculated across the entire segment, that aligned over more than 70% of the length of the fragments were considered homologous and used for further analysis (Goris et al., 2007). The percentage identity values obtained for the fragments were then averaged across all the fragments to obtain the ANI values between the two genomes (Goris et al., 2007). For the *Pantoea* dataset four isolates of *Pan. ananatis*, including the type strain, was compared to the genome of the type strain of *Pan. allii*. The ANI values for the *Paraburkholderia* isolates were obtained from published data (Steenkamp et al., 2015). For the *Escherichia* dataset two representatives for each of the Clades III, IV and V were compared to the type strain of *E. coli*.

References

- GORIS, J., KONSTANTINIDIS, K. T., KLAPPENBACH, J. A., COENYE, T., VANDAMME, P. & TIEDJE, J. M. 2007. DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. *International Journal of Systematic and Evolutionary Microbiology*, 57, 81-91.
- RICHTER, M. & ROSSELLÓ-MÓRA, R. 2009. Shifting the genomic gold standard for the prokaryotic species definition. *Proceedings of the National Academy of Sciences*, 106, 19126-19131.
- STEENKAMP, E. T., VAN ZYL, E., BEUKES, C. W., AVONTUUR, J. R., CHAN, W. Y., PALMER, M., MTHOMBENI, L. S., PHALANE, F. L., SEREME, T. K. & VENTER, S. N. 2015. *Burkholderia kirstenboschensis* sp. nov. nodulates papilionoid legumes indigenous to South Africa. *Systematic and Applied Microbiology*, 38, 545-554.

Genomes used

Isolate	Accession
<i>Pantoea ananatis</i> LMG 2665 T	JMJJ00000000
<i>Pantoea ananatis</i> LMG5342	HE617160.1 HE617161.1
<i>Pantoea ananatis</i> AJ13355	AP012032.2 AP012033.1
<i>Pantoea ananatis</i> B1-9	CAEI00000000
<i>Pantoea allii</i> LMG 24248 T	MLFE00000000
<i>Paraburkholderia kirstenboschensis</i> KB 15 T	JRZC00000000
<i>Paraburkholderia kirstenboschensis</i> Rau2d2	JRTU00000000
<i>Paraburkholderia caledonica</i> NBRC 102488	BAYE00000000
<i>Escherichia coli</i> DSM 30083 T	AGSE00000000
<i>Escherichia</i> Clade III TW09231	AEJW00000000
<i>Escherichia</i> Clade III TW09276	AEJV00000000
<i>Escherichia</i> Clade IV TW14182	AEJZ00000000
<i>Escherichia</i> Clade IV H605	Broad Institute
<i>Escherichia</i> Clade V E1118	Broad Institute
<i>Escherichia</i> Clade V TW09308	AEME00000000

Average Nucleotide Identity (ANI) values calculated with JSpecies between four *Pan. ananatis*

	<i>Pan. ananatis</i> AJ13355	<i>Pan. ananatis</i> LMG 5342	<i>Pan. ananatis</i> B1-9	<i>Pan. ananatis</i> LMG 2665	<i>Pan. allii</i> LMG 24248
<i>Pan. ananatis</i> AJ13355	---	99	99.19	99.23	87.75
<i>Pan. ananatis</i> LMG 5342	99.07	---	99.12	99.04	87.74
<i>Pan. ananatis</i> B1-9	99.22	99.1	---	99.21	87.79
<i>Pan. ananatis</i> LMG 2665	99.22	98.97	99.18	---	87.69
<i>Pan. allii</i> LMG 24248	88.12	88.1	88.11	88.21	---

isolates and the type strain of *Pan. allii*

Average Nucleotide Identity (ANI) values calculated with JSpecies between two *Par. kirstenboschensis* isolates and *Par. caledonica*

	<i>Par. kirstenboschensis</i> KB15	<i>Par. kirstenboschensis</i> Rau2D2	<i>Par. caledonica</i> NBRC102488
<i>Par. kirstenboschensis</i> KB15	---	96.37	92.11
<i>Par. kirstenboschensis</i> Rau2D2	97.18	---	91.98
<i>Par. caledonica</i> NBRC102488	92.65	91.76	---

Average Nucleotide Identity (ANI) values calculated with JSpecies between the *E. coli* type strain and representatives of each of the *Escherichia* clades

	<i>E. coli</i> DSM30038	Clade III TW09231	Clade III TW09276	Clade IV H605	Clade IV TW14182	Clade V E1118	Clade V TW09308
<i>E. coli</i> DSM30038	---	91.71	91.77	92.13	91.95	90.68	90.87
Clade III TW09231	91.82	---	98.39	96.4	96.3	92.08	92.15
Clade III TW09276	91.92	98.31	---	96.45	96.31	92	92.01
Clade IV H605	92.27	96.3	96.37	---	98.96	91.96	91.95
Clade IV TW14182	92.24	96.29	96.32	99.06	---	91.95	91.97
Clade V E1118	90.83	92.06	92.04	92.04	91.89	---	99.37
Clade V TW09308	90.89	92.09	92.02	91.95	91.88	99.3	---